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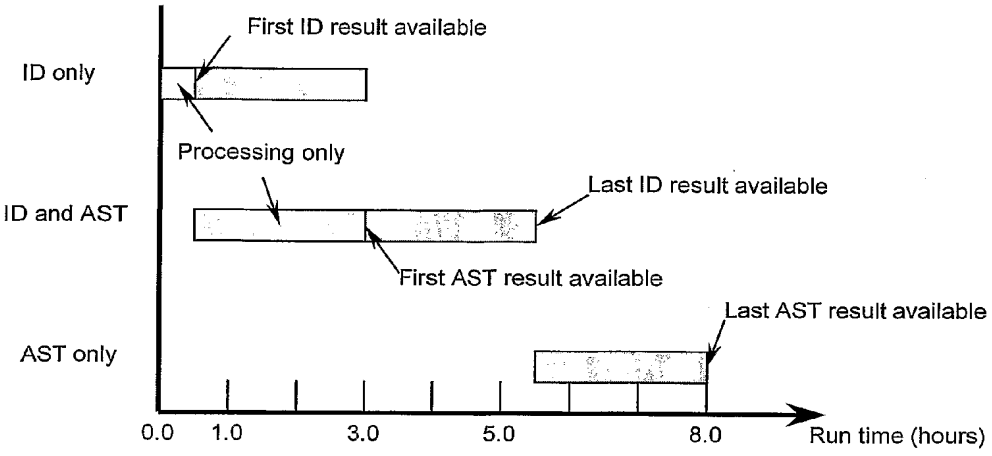


(57) Abstract: Apparatus for microbiological analysis of a patient sample, comprising: a microorganism extractor; a temporary storage facility; a microorganism identifier; an antimicrobial tester; a thermostatically-controlled incubator; a timer; and sample router(s), for routing: patient sample to the microorganism extractor; extracted microorganisms to the temporary storage facility; microorganisms from the temporary storage facility, patient sample or extracted sample to the microorganism identifier; and microorganisms from the temporary storage facility to the antimicrobial tester. The invention allows microorganism identification without prior need for sample culture, thus giving a significant reduction in the time required between taking a sample and identifying an appropriate antimicrobial therapy. Furthermore, where organisms are identified after their extraction, the invention allows a single non-specific technique to be used for specifically identifying different microorganisms, thus simplifying overall analysis. In addition, results of the identification and antimicrobial testing assays can be analysed by a common technique, which again simplifies the overall analysis.

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# SYSTEM FOR RAPID MICROORGANISM IDENTIFICATION AND ANTIMICROBIAL TESTING

All documents cited herein are incorporated by reference in their entirety.

## TECHNICAL FIELD

This invention is in the field of clinical diagnostic microbiology.

## BACKGROUND ART

Infectious diseases are the second largest cause of death worldwide and are the biggest killers of children. Antibiotics are generally useful for treating bacterial infections, but antibiotic resistance is on the increase.

When a patient has a bacterial infection, a physician needs to know how best to treat it, and the best treatment will depend on the antibiotic susceptibility of the bacterium in question. Identification of the bacterium and of its antibiotic susceptibility can be performed in clinical laboratories but, despite the high global human and financial cost of infectious diseases, many tests for pathogenic bacteria still use the labour-intensive methods developed by Pasteur in the 19th century. In these tests, bacteria from the patient are grown on plates in specialised culture media until they reach sufficient numbers to be seen by the human eye. This culture step takes 24-48 hours and, once the bacteria have been identified, it takes a further 24 hours before antibiotic susceptibility can be determined.

From taking a patient sample to identifying an appropriate antimicrobial therapy therefore takes 2 to 3 days. Current infectious disease diagnostics therefore have little impact on patient management. As a physician cannot afford to wait 3 days before starting treatment, patients are subjected to 'best guess' antimicrobial therapy, and this typically involves expensive broad-spectrum antimicrobials which may be unnecessary or inappropriate. Hospital stays are increased in length, leading to higher costs, and there is also an increased risk that antimicrobial resistance will develop. When laboratory results arrive, they are generally used to confirm whether the 'best guess' was appropriate rather than being used to educate or inform clinical choices.

More rapid methods for identifying bacteria have been developed. PCR, for instance, is very rapid and, with the correct primers, is very sensitive and precise. Furthermore, multiplex PCR methods can identify several bacteria in parallel in a single sample. However, the PCR method is not so useful for testing antimicrobial susceptibility as it relies on detecting the presence of known antimicrobial resistance genes and so requires *a priori* knowledge of those resistance genes. Furthermore, resistance may arise from a wide variety of diverse mechanisms, each of which may be encoded by different genes or mutations in isolation or in combination, and the contribution of each mechanism to overall antimicrobial resistance is difficult to determine by genetic testing. Genotypic testing for AST therefore cannot compare to phenotypic testing and has limited practical value in the clinical diagnostic setting.

It is an object of the invention to provide improvements in clinical diagnostic microbiology. In particular, it is an object to provide systems for both rapid identification and rapid antimicrobial sensitivity testing of clinically-important bacteria, direct from clinical samples.

## DISCLOSURE OF THE INVENTION

The invention provides an apparatus for microbiological analysis of a patient sample, comprising: (a) a microorganism extractor, for extracting one or more microorganism(s) from the patient sample to give one or more extracted microorganism sample(s); (b) a temporary storage facility, for storing extracted microorganism sample(s); (c) a microorganism identifier, for identifying one or more specific microorganism(s) within the patient sample and/or within the extracted microorganism sample(s); (d) an antimicrobial tester, for determining the effect of one or more antimicrobial(s) on micro-organism(s) within extracted microorganism sample(s) and/or within the patient sample; (e) a thermostatically-controlled incubator for incubating extracted microorganism sample(s); (f) a timer; and (g) one or more sample routers, for routing: patient sample to the microorganism extractor; extracted microorganisms to the temporary storage facility; microorganisms from the temporary storage facility, patient sample or extracted sample to the microorganism identifier; and microorganisms from the temporary storage facility to the antimicrobial tester.

The invention also provides a process for analysing a patient sample, comprising the steps of: (a) identifying microorganisms present within the patient sample; (b) extracting one or more microorganisms from the patient sample, to give extracted microorganism sample(s); and (c) determining the effect of one or more antimicrobial(s) on extracted microorganism sample(s) or on the patient sample. Steps (a) and (b) are performed prior to step (c) but, depending on the particular identification technique being used, steps (a) and (b) can occur in either order. In addition, step (b) may be performed multiple times, both before and after step (a). The steps of the process may advantageously be performed within a single apparatus, without the need for user intervention.

Advantageously, the invention allows microorganism identification without prior need for plate culture of the patient sample, thus giving a significant reduction in the time required between taking a patient specimen and identifying an appropriate antimicrobial therapy. The invention thus allows rapid testing of samples by avoiding the rate-limiting step in current clinical diagnostic methods.

Furthermore, where organisms are identified after their extraction, the invention allows a single non-specific technique to be used for specifically identifying different microorganisms, thus simplifying overall analysis.

A further advantage of the invention is that results of the microorganism identification assay and the antimicrobial testing assays can both be analysed by a common technique, which again simplifies the overall analysis procedure.

The invention also provides a process for performing microbial identification and antimicrobial susceptibility testing on a patient sample, wherein microbial identification is performed without culture of microorganisms. Similarly, the invention provides an apparatus for performing microbial identification and antimicrobial susceptibility testing on a patient sample, wherein microbial identification is performed without culture of microorganisms within the apparatus.

The invention also provides a method for selecting an antimicrobial treatment for a patient, comprising the steps of: (a) taking a sample from the patient; (b) sending the sample for analysis to determine the effect of one or more antimicrobial(s) on micro-organisms in the sample, with optional identification of micro-organisms present in the sample; and (c) receiving for the results of step (b), which results indicate one or more antimicrobial(s) that is/are effective against a micro-organism in the sample. The time which elapses between taking the sample in step (a) and receiving the results in step (c) is generally less than $x$ hours, where the value of $x$ is selected from 72, 48, 36, 24, 12, 8, 7, 6, 5 or 4. The method will generally be a method for treating a patient, comprising the further step of (d) administering to the patient one or more of the antimicrobial(s) indicated in step (c). The time which elapses between taking the sample in step (a) and administering the antimicrobial(s) in step (d) is also generally less than $x$ hours.

Thus a physician is able to rationally select an effective treatment for the patient based on the results of antimicrobial testing, rather than embarking on an antimicrobial treatment 'blind' before the results are available, which is a significant advantage arising from the speed of the process of the invention. This method of the invention can be most readily achieved where the analysis in step (b) occurs without requiring culture of microorganisms prior to antimicrobial testing steps and/or micro-organism identification steps.

The invention also provides a process for performing antimicrobial susceptibility testing on a sample taken from a patient, wherein the time that elapses between taking the sample from the patient and providing the results of antimicrobial susceptibility testing is less than $x$ hours. Similarly, the invention provides a process comprising the steps of (a) taking a sample from a patient, (b) performing antimicrobial susceptibility testing on the sample, and (c) identifying at the end of step (b) one or more antimicrobial(s) that is/are effective against micro-organisms present in the sample, wherein the time that elapses between taking the sample in step (a) and identifying an antimicrobial in step (c) is less than $x$ hours. The value of $x$ is as described above.

***The patient sample***

Specimens taken from patients (*e.g.* blood, stools, swabs, mucous, tissue, *etc.*) are generally not suitable for direct microbiological testing, and so have to be processed (*e.g.* liquefied, diluted, separated) to give samples for use with the invention. Some specimens, however, such as urine or cerebrospinal fluid can be used directly as samples with the invention, without processing. Where it is required, specimen processing will generally be performed prior to analysis according to the invention, although it is also possible for an apparatus of the invention to include a sample processor for processing direct specimens into a form suitable for testing.

The term 'patient sample' therefore includes both material taken directly from a patient and material obtained by processing material taken directly from a patient (*i.e.* indirectly obtained from the patient, *e.g.* a blood culture). In some embodiments, it includes a microorganism plate culture

obtained from material taken from a patient. Advantageously, however, the invention avoids the prior need for such organism plate culture.

For some types of sample where microorganism numbers are low (*e.g.* in swabs) a short incubation step to increase microorganism numbers may advantageously be used for increasing sensitivity, but this is not essential. This incubation step will generally take place after microorganism extraction.

***Microorganism extraction***

The invention requires the extraction of microorganism(s) from a sample, to give one or more extracted microorganism samples. Extracted microorganism samples can then be subjected to procedures for microorganism identification and/or antimicrobial testing. Without prior extraction of microorganisms, the results of such procedures are not easy to interpret.

There are three principal routes by which extracted microorganism samples can be prepared ready for use in microorganism identification and then in antimicrobial testing: (1) one extraction is performed on a patient sample, to give an extracted microorganism sample, which is then split to give a first extracted microorganism (sub)-sample for use in microorganism identification and a second for antimicrobial testing; (2) separate extractions are performed on the same patient sample, to give a first extracted microorganism sample for use in microorganism identification and a second for use in antimicrobial testing; (3) a patient sample is divided, with a first sub-sample being subjected to extraction to give microorganisms for identification and a second sub-sample being subjected to extraction to give microorganisms for antimicrobial testing. It is preferred that the same route is used for each microorganism to be extracted *e.g.* all microorganisms are extracted via route (2), rather than using route (1) for streptococci and route (3) for meningococci, *etc.* However, the overall scheme shown in Figure 6 is followed.

Where route (2) or (3) is used, the two extractions can be performed at separate times. Where the second extraction is timed after microbial identification has been performed, the invention thus advantageously allows the second extraction to be optional for a particular microorganism, with extraction depending on a positive identification result. For example, if a first *S.pneumoniae* extraction is performed on a sample in route (2), but the result of the extraction is negative, then there is no need to perform a second *S.pneumoniae* extraction for antimicrobial testing. Routes (2) and (3) therefore allow the results of microorganism identification to educate antimicrobial testing and minimise wasteful extraction steps. [NB: although this passage refers to "two" extractions, it will be appreciated that the figure "two" refers to the eventual fate of the extracted material, rather than implying that only "two" extractions can physically take place. Thus the "two" extractions in (2) could involve many more than two physical acts of extraction, and the patient sample could be divided in (3) to give many more than two sub-samples, but the extracted material may have a first fate (identification) or a second fate (antimicrobial testing).].

It is important to the invention that the extraction technique which is used should not kill the microbe, as antimicrobial testing must be performed on living microorganisms in order to give a useful result. Although identification can be performed on dead microorganisms, using a lethal extraction technique for identification purposes and a non-lethal technique for susceptibility testing purposes is more complicated than using a common non-lethal technique for both purposes. Furthermore, the use of a non-lethal technique for extraction allows the extracted microorganisms to be used for purposes other than antimicrobial testing.

It is also preferred that the extraction technique should not inhibit growth of the microbe as antimicrobial testing requires multiplying or growing microorganisms, and reversing the growth inhibition before commencing identification is more complicated than necessary.

Various methods are available for extracting microorganisms of interest, and any of these methods can be used with the present invention. For specificity, extraction methods generally rely on immunochemistry, using an antibody for a microorganism-specific antigen. A preferred technique for use with the invention is based on immunomagnetic separation (IMS) methods, in which magnetic particles (typically beads) are coupled to antibodies specific for antigens on the surface of microorganisms of interest [refs. 1 & 2]. The antibodies interact with microorganisms to form particle-microorganism complexes. These complexes can then be separated by the use of magnets. Organisms may also be extracted by techniques such as: the use of flow cytometry of cell sorting based on fluorescent labelling (*e.g.* FACS); differential filtration (*e.g.* based on physical or chemical characteristics of the cell, membrane affinity, tunable membranes, *etc.*); dielectrophoresis; capture based on cell-surface molecules such as ligand or lectin based capture; non-antibody receptors, such as recombinant phages or other combinatorial peptides; *etc.* In general, therefore, any physical, immunological or chemical means of extraction can be used, and the choice of extraction technique will depend on factors such as cost, convenience, sample type (*e.g.* some techniques are better for blood than others), desired specificity, *etc.*

According to the invention, a plurality of microorganisms can be extracted from samples (*e.g.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more), although in some embodiments it may be desired to extract only a single organism. Different microorganisms may be extracted together or simultaneously in parallel, but it is more typical to extract them separately in series, particularly where immunomagnetic methods are used. The apparatus of the invention may therefore comprise a supply of plurality of microorganism-specific extraction reagents for delivering to the microorganism extractor. These can be introduced into a sample individually and then processed to extract microorganisms in series.

It will be appreciated that a microorganism can only be "extracted" if it is present in a sample. References to "extracting" a microorganism should therefore be interpreted as referring to the potential to do so if the microorganism is present. Thus the invention could be used to "extract" microorganisms from a sample which contains no microorganisms (*e.g.* a sterile sample), but none of

the extraction steps performed on the sample would actually result in any microorganisms being removed. Nevertheless, the sample has been subjected to microorganism "extraction".

***Microorganisms***

The invention can specifically extract a plurality of different microorganisms from within a patient sample. The degree of specificity in the extraction may depend on the needs of an individual user. Taking streptococci as an example: it may be desired to extract all streptococci in a sample; it may be desired to extract individual species (*e.g.* separate extraction of *S.pneumoniae* and *S.agalactiae*); it may be desired to extract particular serotypes (*e.g.* separate extraction of serotypes 6B, 14, 19, 23F of *S.pneumoniae*); or it may be desired to extract particular strains (*e.g.* separate extraction of penicillin-resistant and penicillin-sensitive strains of *S.pneumoniae*). At a higher level, it may be desired to extract all bacteria or all yeast, depending on the user's needs. At all of these levels, however, the extraction is "specific" in the sense that microorganisms of interest are extracted but other microorganisms are left in the sample.

The invention preferably involves the extraction of bacteria and/or fungi and/or parasites from a sample. More preferably, it can extract the following microorganisms: *Staphylococci*, such as *S.aureus* (and more particularly methicillin-resistant *S.aureus*); *Enterococci* such as *E.faecium* and *E.faecalis* (and more particularly vancomycin-resistant *E.faecalis*); *Streptococci* such as *S.pyogenes*, *S.pneumoniae* (and more particularly penicillin-resistant *S.pneumoniae*), and *S.agalactiae*; Coliforms such as *E.coli*, *Klebsiella* species, *Proteus* species, and *Enterobacter* species; Enteric organisms like *Salmonella* species, *Shigella* species, and *Campylobacter* species; *Neisseria* species such as *N.meningitidis*, *N.gonorrhoeae*; yeasts, such as *C.albicans*; parasites such as *P.falciparum*, *Leishmania*; spirochaetes; schistosoma; and specific pathogens such as *Burkholderia cepacia*, *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Corynebacterium diphtheriae* and *Bordetella pertussis*. This list is not exhaustive, but serves to illustrate the wide range of disease-causing microorganisms which can be detected using the invention.

Different organisms typically have different optimum growth conditions (media, aerobic/anaerobic, temperature, *etc.*). For example, streptococci grow well in Todd-Hewitt medium, whereas *S.aureus* prefers peptone. The invention may thus utilise a number of different conditions but, for simplicity, it is preferred to compromise by using 'generic' media *e.g.* BHI (brain heart infusion). Where the invention involves antimicrobial sensitivity testing of a specific organism, however, it requires a choice of conditions which allows some growth of that organism *e.g.* it may use a common medium for all microorganisms except for one, which requires a specific medium. The choice of growth medium will ultimately depend on the choice of microorganisms to be detected and such choices are familiar to workers in this field. The choice of growth medium may depend on geographical location *e.g.* the EU and USA have different standard methodologies.

***Organism identification***

The invention involves the identification of microorganisms within a patient sample. Identification techniques may be performed on the patient sample itself, or on extracted microorganism samples.

If a technique is used which operates on the patient sample itself, it is preferred to use a technique which can identify specific microorganisms in the presence of other microorganisms. Gram-staining would not be suitable, for instance, but microorganism-specific PCR would be suitable. Multiplex PCR can be used in order to identify specific microorganisms in parallel.

Techniques which operate on the sample itself are not preferred, however, as target microorganisms still have to be separated from other microorganisms prior to antimicrobial testing – there is no real advantage in using parallel techniques for identification in mixtures of microorganisms when separation is eventually necessary anyway.

It is therefore preferred that the microorganism identifier should be used after microorganisms have been extracted. Advantageously, this allows the apparatus to use a non-specific microorganism identification technique. For example, if *E.faecalis* and *S.agalactiae* have each been specifically extracted from a sample, it is not necessary to use different microorganism identification techniques on the two extracts – any technique which non-specifically identifies the presence of a bacterium will give the necessary information. If *E.faecalis* is specifically extracted from a sample then the presence of a bacterium in the extract means that it must be *E.faecalis*. Thus a non-specific test still gives a microorganism-specific result because of prior microorganism-specific extraction. The apparatus can therefore use the same non-specific test on all extracted bacteria. However, the use of specific detection methods is not excluded, even where specific extraction has been performed. For instance, IMS followed by PCR has been reported for organism identification in patient samples [*e.g.* refs. 3 to 7, *etc.*]

Non-specific methods for identifying (detecting) bacteria include staining (*e.g.* Gram staining), fluorescence (*e.g.* actin orange), flow cytometry, *etc.*

Preferred methods report results optically *e.g.* by luminescence. A preferred assay for detecting microorganisms relies on the release of adenylate kinase (AK) after cell lysis [*e.g.* refs. 8 to 12] and the subsequent production of ATP from exogenous ADP, with the ATP being used to drive a luciferase reaction. This assay is more than 100-fold more sensitive than use of endogenous ATP to drive the luciferase reaction and also shows better correlation with cell numbers. Furthermore, the AK assay is at least as sensitive as PCR for bacterial detection.

Thus the microorganism identifier preferably utilises a method involving lysing microorganisms in the sample (*e.g.* by the use of detergent, sonication, a bacteriolysin, or a phage, which phage may be specific to a particular microorganism), adding highly-pure ADP and a source of $Mg^{2+}$ ions to a sample and determining the amount of ATP produced from the ADP. The molar concentration of $Mg^{2+}$ ions is preferably the same as or greater than the molar concentration of ADP such that all ADP

molecules can be associated with at least one $Mg^{2+}$ ion. The method may also involve adding luciferin and luciferase to the sample during or, preferably, after the generation of ATP from ADP by adenylate kinase, followed by determining the amount of luciferase-generated light emitted from the sample. Further details of the method are disclosed in references 8 to 12.

In general, the invention can use a general extraction method followed by a specific identification method, or a specific extraction method followed by a general identification method. If several bacteria are extracted together, they will be more specifically identified later; if a specific extraction is used, a general microorganism detection method may be used. The former methods is most useful where it is known that a sample is either infected with microorganism X or it is not and the goal is to find those patients who have been infected *i.e.* a specific extraction is not required because there is only one possible target. Both methods allow microorganisms to be identified with specificity.

***Antimicrobial testing***

The invention involves testing one or more antimicrobials on microorganisms present within a patient sample. Antimicrobial testing typically requires the microorganisms to have been separated from any other microorganisms within the sample and so is preferably performed after a microorganism extraction. In some situations, however, antimicrobial testing can usefully be performed before microorganism extraction (*e.g.* a non-specific or 'global' antimicrobial test). Antimicrobials may also be added before extraction such as when the goal is a yes/no test for the presence of a specific organism and the antimicrobial is used to enrich the target organism selectively

To minimise waste, it is preferred to base antimicrobial testing on the results of microorganism identification. In particular, antimicrobial testing need only be performed against microorganisms which have given a positive result in identification. For example, if ten bacteria are extracted from a patient sample and only three are present in the extracts, antimicrobial testing would only be performed on extracted samples for those three bacteria. Testing extracted samples for the other seven bacteria would evidently be wasteful. In some situations, negative extraction results may mean that antimicrobial testing is not even performed (*e.g.* if the sample is tested to identify the presence only of MRSA, but the bacterium is absent, the result of the test is simply 'negative' and there is no need to test antimicrobial susceptibility).

Preferably, therefore, antimicrobial testing is performed only on extracted microorganism samples which have given a positive identification result, and not on extracted samples which do not contain any microorganisms. However, in some embodiments it may be desired (*e.g.* for ease of automation) to perform antimicrobial testing on all extracted microorganism samples regardless of the results of microorganism identification.

A second way of using the results of microorganism identification to educate antimicrobial testing is to select particular panels of antimicrobials for testing. If a penicillin-resistant and

amoxycillin-resistant bacterium is identified, for example, the antimicrobials which are subsequently tested against that bacterium can be adapted accordingly.

Where the microorganism identification technique was non-lethal, antimicrobial testing can be performed on the same microorganisms as used for identification. In general, however, the identification technique used is lethal (*e.g.* the AK-based techniques, which require cell lysis). More than one extracted sample is therefore required, and these can be prepared by any of the three routes (1), (2) or (3) as described above, with route (2) being preferred.

A preferred antimicrobial testing technique is AST (antimicrobial susceptibility testing [*e.g.* refs. 13 to 15]). Essentially, this technique involves incubating a microorganism in the presence of a number of different antimicrobials in order to determine which antimicrobial(s) can inhibit the growth of the microorganism and thus be suitable for patient treatment. Another antimicrobial test which may be performed is the generation of a killing curve, in which the effect of an antimicrobial at a given concentration is followed over time. The effect of antimicrobials on growth can be assessed in many ways, including the use of quantitative PCR to assess cell numbers based on DNA content [16,17].

If antimicrobials are tested at various concentrations, the technique can be used to identify minimum inhibitory concentration (MIC) values for antimicrobials (*i.e.* the lowest concentration of a particular antimicrobial which can inhibit the growth of a given microorganism) or minimum bactericidal concentration (MBC) values (*i.e.* the lowest concentration which can kill a given microorganism).

According to the invention, a plurality of antimicrobials can be tested (*e.g.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more). The apparatus of the invention may therefore comprise a supply of a plurality of antimicrobials for delivery to the antimicrobial tester. The user may be able to choose the number of antimicrobials to be tested.

Furthermore, a plurality of concentrations of each antimicrobial can preferably be tested (*e.g.* 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more), and preferably between 4 and 8 (*e.g.* 6). It is preferred to test a range of concentrations in the range from 0.05 to 50 mg/ml, more preferably a range from 0.125 to 16 mg/l. The range preferably spans the known break-point for an antimicrobial. The apparatus may store the antimicrobial in a range of pre-determined concentrations and/or may prepare dilutions as necessary from a stock solution. The user may be able to choose the concentrations to be tested.

Thus a user may be able to choose the antimicrobial(s) and range of concentrations to be tested to allow them to customise the test appropriate to their needs.

Advantageously, the results of antimicrobial testing are reported in the same way as the results of microorganism identification such that a single detection mechanism can be used to determine the outcome of both assays. It is therefore preferred that a luminescence method is used for the antimicrobial testing. A method based on the use of adenylate kinase is reported in reference 18, and a method based on the use of flow cytometry is reported in reference 19.

When sensitive assay methods are used (*e.g.* the use of adenylate kinase, which is more than 100-fold more sensitive than use of endogenous ATP to drive the luciferase reaction) then positive results can be achieved with low numbers of cells, which means that plate culture of micro-organism growth can be avoided. A sample can instead be directly used for identification and/or antimicrobial testing (*e.g.* AST, MBC, MIC, *etc.*) which allows results to be obtained much more quickly than when conventional techniques are used. Avoidance of slow steps, such as micro-organism growth, allows results to be generated rapidly, which in turn means that a physician can choose an early antimicrobial treatment based on patient-specific diagnostic data rather than on a hunch.

***Antimicrobials***

The invention involves the use of antimicrobials. The term "antimicrobial" refers to any substance (typically an organic compound) that can kill, or inhibit the growth of, a microorganism. The term includes natural and synthetic compounds. It includes antibiotics and antimycotics within its scope, with antibiotics being a preferred subset of antimicrobials.

Classes of antimicrobials which may be tested include beta-lactams, aminoglycosides, fluoroquinolones, sulfonamides, glycopeptides, carbapenems, azoles, oxazolidinones, macrolides, quinolones, tetracyclines, *etc.* The invention can also be used to test phages [20] and bacteriolysins.

Typical antimicrobials for use with the invention are: penicillin, amoxycillin, ciprofloxacin, cephalothin, ampicillin, augmentin, linezolid, gentamicin, flucluxacillin, vancomycin, chloramphenicol, tetracycline, minocycline, sulfonamide, oxazolidinone, fluconazole, nitrofurantoin, trimethoprim, nalidixic acid, amphotericin, kanamycin, streptomycin, *etc.*

The invention may also be used to test the effect of mixtures of two or more antimicrobials. Testing combinations may identify positive or negative synergies between the antimicrobials against a particular extracted microorganism.

Different antimicrobials typically have different activity profiles *e.g.* they may be slow- or quick-acting. Each antimicrobial test may therefore be different. As the invention involves the use of known antimicrobials, however, the invention can be adapted according to the profile of any particular antimicrobial. Antimicrobial testing will typically last between 15 minutes and 4 hours. Reading results at around 2 hours is generally convenient.

***Apparatus of the invention***

The apparatus of the invention includes a microorganism extractor which is used to extract microorganism(s) from a patient sample. Where microorganism extraction requires specific reagents, the apparatus preferably includes a supply of those reagents together with means for delivering them to the microorganism extractor. The apparatus will also comprise means for using the reagents to extract microorganisms.

For example, the apparatus of the invention may include a supply of immunomagnetic particles (preferably a supply of a plurality of different immunomagnetic particles which recognise different

specific microorganisms) and a magnet. Similarly, it may include a supply of fluorescently-labelled antibodies (preferably a supply of a plurality of different labelled antibodies which recognise different specific microorganisms) and a fluorescence-activated cell sorter.

The apparatus preferably includes a shaker *e.g.* to prevent settling of IMS particles in suspension.

The apparatus includes storage means for storing extracted microorganisms prior to downstream processing. Microorganisms are thus transferred from the patient sample into temporary storage means. They may then be taken from the temporary storage means for identification and/or antimicrobial testing.

The apparatus of the invention includes a microorganism identifier, which is used for identifying specific microorganism(s) from within the patient sample. It may act on the patient sample itself or it may act on an extracted microorganism sample (*e.g.* as stored in the temporary storage means). Where microorganism identification requires specific reagents, the apparatus preferably includes a supply of those reagents together with means for delivering them to the microorganism identifier. The apparatus will also comprise means for using the reagents to detect microorganisms.

For example, the apparatus of the invention may include a supply of lysis reagent (*e.g.* a detergent or a bacteriolysin) and/or a lysis mechanism (*e.g.* a sonicator). In preferred embodiments of the invention, the apparatus includes supplies of $Mg^{2+}$, ADP, luciferin and luciferase. Where bioluminescent reagents are used, the apparatus should also include a dark or substantially light-sealed compartment. The reagents may be stored in opaque containers.

It is further preferred that the apparatus includes one or more luminometers. Furthermore, it is preferred that the apparatus should include a means for calibrating the luminometers.

Where other optical detection methods are used, the apparatus may include, for instance, a laser or a cell-sorter. The apparatus may include a turbidity detector, a spectrophotometer, a pH monitor, *etc.*

It is preferred that the same reagents can be used to identify a plurality of different microorganisms with the same microorganism identifier.

The apparatus of the invention includes an antimicrobial tester, which is used for determining the effect of one or more antimicrobial(s) on micro-organism(s) within extracted microorganism samples (*e.g.* as stored in the temporary storage means). The apparatus preferably includes a plurality of antimicrobials together with means for supplying the antimicrobials to the antimicrobial tester. The apparatus will also comprise means for determining the effect of antimicrobials on microorganisms. Antimicrobials are typically stored in dehydrated form. Conveniently, antimicrobials can be stored in a plurality of predetermined concentrations.

The effect of antimicrobials is typically determined by incubating microorganisms in their presence. The incubation should be carried out under controlled conditions so as to permit standardisation. Thus the apparatus of the invention comprises a temperature-controlled incubator (*e.g.* controlled

using a thermostat, at 37±2°C). The apparatus may also include a supply of one or more (sterile) growth media (*e.g.* BHI) together with means for delivering them to the antimicrobial tester. A wash broth and a growth broth will typically be included. The apparatus may also include a supply of "stop solution" for preventing further growth of bacteria beyond a specific time-point.

Advantageously, the apparatus of the invention uses the same hardware for implementing both microorganism identification and antimicrobial testing. For example, the two distinct aspects of the apparatus may share routers, carousels, timers, *etc.*

The apparatus can preferably receive a plurality of patient samples for individual processing, with samples being held in a queue before they reach the microorganism extractor. The apparatus may also include a queue bypass mechanism to allow a particular patient sample (*e.g.* for reasons of urgency) to be analysed as soon as possible.

The apparatus preferably includes a waste disposal unit for receiving samples after processing, including receiving material from spent identification and antimicrobial testing tests. This unit may include a strong antimicrobial (*e.g.* disinfectant or bleach) to prevent microorganism growth. Disinfectants may also be used for cleaning the apparatus mechanisms (*e.g.* following an HIV test, or prior to engineer testing), or the apparatus may include a steam generator for pasteurisation.

The apparatus will usually include a volumetric dispenser, for removing and delivering accurate volumes from the various liquid materials which are handled during the analysis procedure.

The apparatus may include a refrigeration mechanism *e.g.* for storage of culture medium or broth, bioluminescence reagents, *etc.*

The apparatus may include a rehydration mechanism *e.g.* where dehydrated antimicrobials are used. The apparatus may also include a supply of buffer or water *e.g.* for rehydration or for washing.

The apparatus includes a timer, for monitoring extractions, identifications, incubations, *etc.*

The one or more sample routers, by which samples and microorganisms are moved around the apparatus in the correct sequence for microbial identification and antimicrobial testing, are a key feature of the apparatus of the invention, and they are preferably automated rather than being manual. To aid automated routing, samples may be labelled within the apparatus *e.g.* their containers may display bar-codes, and the apparatus would thus include a bar-code scanner.

The apparatus is preferably controlled by a computer. The computer is programmed to co-ordinate the introduction of samples to the microorganism extractor, the transfer of samples to the a microorganism identifier; and the transfer of samples to the antimicrobial tester. It is also programmed to co-ordinate procedures taking place within the individual elements of the apparatus *e.g.* by controlling automated sample routers. The timer is preferably part of the computer.

The apparatus will typically include a visual display *e.g.* to indicate internal status of the apparatus, user instructions, *etc.* It may include a printer for producing hard copies of results.

The apparatus preferably includes mechanisms for monitoring the remaining supplies of consumables (solutions, reaction vessels, *etc.*) and ways of alerting users to low supplies.

In general, components of the apparatus will be sterile.

The various components of the apparatus of the invention are generally integrated into a single device. During use the integrated apparatus will present a user interface and a place for loading samples but the remaining operating parts can be hidden from view.

***Process of the invention***

The process of the invention involves a step of extracting specific microorganisms from a patient sample. In preferred embodiments the invention uses IMS to perform the extraction. The process of the invention may therefore involve one or more of the following steps: (a) mixing immunomagnetic particles with a patient sample, wherein the particles comprise an antibody which specifically binds to a target microorganism; (b) allowing the sample to interact with the particles; (c) placing the sample in a magnetic separator which causes the magnetic particles to separate from the sample; (d) aspirating liquid from the sample to leave the particles, without removing bound microorganisms; and (e) washing the particles with a solution *e.g.* to remove preservative, non-specific binding substances and loosely bound bacteria. Step (b) preferably lasts anywhere between 0.5 seconds and 180 minutes, depending on the concentration of microorganisms in the sample, the concentration desired for further work, and the extraction technique being used. For example, it may last at least 1, 2, 3, 4, 5, 10, 15, or 30 minutes. It may last less than 60, 45, 30, 20, 15, 10, 9, 8, 7, 6 or 5 minutes. A preferred period for step (b) is around 30 minutes *e.g.* when IMS is used.

The process of the invention involves a step of identifying microorganisms within the sample. This step may be performed on the patient sample or it may be performed on a microorganism sample derived from the patient sample (*e.g.* an extract stored in the temporary storage means). Identification of a plurality of microorganisms may be performed in parallel. This may be inherent in the technique used (*e.g.* multiplex PCR within a patient sample) or separate reactions may be performed together (*e.g.* simultaneous luminescent detection of several extracted microorganism samples).

In preferred embodiments the invention uses an AK-based assay for the identification. The process of the invention may therefore involve the steps of lysing microorganisms present in a sample, followed by determining the levels of released AK. AK levels are preferably determined by adding $Mg^{2+}$, ADP, luciferin and luciferase to the sample and then determining the amount of emitted light.

The process of the invention involves a step of determining the effect of one or more antimicrobial(s) on microorganisms which have been extracted from the sample. This step will typically be preceded by a step of splitting an extracted microorganism sample into a plurality of extracted microorganism sub-samples. The individual sub-samples can then be subjected to individual antimicrobial testing.

Sub-samples are preferably tested in parallel. For example, once a first antimicrobial test is started, a second may be started before the first is finished *etc.* This allows the results of several tests to be

obtained more quickly than serial testing. The parallel tests may involve the same antimicrobial at the same concentrations (*e.g.* to check experimental variability), the same antimicrobial at different concentrations (*e.g.* for MIC or MBC assays), or different antimicrobials (*e.g.* for AST assays) at the same or different concentrations.

For a single AST, MIC or MBC test, the process of the invention may involve: adding an antimicrobial at a pre-determined concentration to a sub-sample; incubating the sub-sample in the presence of the antimicrobial for a pre-determined time period; and assessing the number of microorganisms in the sub-sample at the end of said time period.

For a complete AST test, the process of the invention may involve: adding a plurality of different antimicrobials at different pre-determined concentrations to a plurality of different sub-samples; incubating the sub-samples in the presence of the antimicrobials for pre-determined time periods; and assessing the numbers of microorganisms in the sub-samples at the end of said time periods.

For a complete MIC or MBC test, the process of the invention may involve: adding an antimicrobial at a plurality of different pre-determined concentrations to a plurality of different sub-samples; incubating the sub-samples in the presence of the antimicrobial for pre-determined time periods; and assessing the numbers of microorganisms in the sub-samples at the end of said time periods.

For killing curve testing, the process of the invention may involve: adding an antimicrobial at a pre-determined concentration to a sub-sample; incubating the sub-sample in the presence of the antimicrobial for a pre-determined time period; and assessing the number of microorganisms in the sub-sample at a plurality of time points within said time period.

These tests will typically also include a step of determining the number of microorganisms in a sub-sample at time zero.

In general, assessment of microorganism numbers in a sub-sample taken at a specific time will not be performed immediately. A typical process will thus require the inhibition of further growth in a sub-sample once an assessment is to be made. Further growth can be inhibited by addition of "stop solution" such as an azide, by cooling or rapid freezing, by lysis, *etc.*

Assessment of microorganism numbers preferably uses an AK-based assay. The process of the invention may therefore involve the step of lysing any microorganisms present in a sample (*e.g.* by treatment with a lytic agent, or by a physical method such as sonication), followed by determining the levels of AK in the sample. AK levels are preferably determined by adding $Mg^{2+}$, ADP, luciferin and luciferase to the sample and determining the amount of light emitted from the sample. Where an antimicrobial is itself lytic (*e.g.* β-lactam antimicrobials such as penicillins like ampicillin and amoxycillin) a separate lysis step may be avoided. Where lysis is used for preventing bacterial growth beyond a specific time point, the AK detection reaction may be prevented by withholding a vital reactant from the lysate *e.g.* by not adding $Mg^{2+}$.

The incubation step preferably takes place at a predetermined temperature *e.g.* at 37±2°C. Higher temperatures may be used if desired *e.g.* at 41°C the doubling time of *E.coli* is 7 minutes, compared to 20 minutes at 37°C, so higher temperatures can accelerate analysis. Higher temperatures are also useful for some slow-growing organisms. The temperature preferences of different bacteria are well known in microbiology and temperatures used in the invention can be modified accordingly.

Antimicrobial testing will typically be accompanied by a control analysis in which a microorganism is incubated in the absence of antimicrobials.

The process of the invention advantageously uses the same detection reaction and mechanism for the two separate steps of bacterial identification and antimicrobial testing. Use of the same detection technique favours compactness and eases maintenance. It is preferred that both steps (*i.e.* identification and testing) use an AK-based assay in which results are detected by luminescence.

The process of the invention may further comprise the step of using the results of the antimicrobial testing step to calculate a MIC and/or MBC value for a given microorganism in a patient sample. MIC values may be presented as true MICs, abridged MICs, or calculated MICs.

The process of the invention may include control tests. Typical negative controls could be to perform microbial extraction on basic culture medium, to perform AST on basic culture medium, *etc.* Other controls may include luminescence standards *etc.*

The process of the invention preferably utilises an apparatus of the invention.

***Random access antibiotics***

The most widely used AST method is the disc agar diffusion test. The procedure, which is accepted by the National Committee for Clinical Laboratory Standards (NCCLS), is a modification of the Kirby-Bauer test. Antibiotic impregnated discs are put on the surface of an appropriate medium in which bacteria are growing. After incubation, zones of inhibition are measured and can be translated into predetermined categories as susceptible, intermediate, or resistant.

The BBL™ Sensi-Disc™ system from Becton Dickinson is an AST system based on small discs, each of which contains a predetermined amount of a predetermined antimicrobial agent. Individual discs are placed onto a pure culture of microbes grown from clinical samples and, after incubation, zones of inhibition surrounding the discs are measured. A number of discs can be applied to a single growing culture and AST results can be determined. Neo-Sensitabs™ from Rosco is a similar product. ADATAB™ tablets are accurately prepared quantities of antibiotic contained in a bacteriologically inert, non-interfering carrier substance. On addition to molten agar the tablet dissolves and a specific antimicrobial concentration is achieved. The tablets are used for AST. The SENSITITRE™ system from TREK Diagnostics uses 96 well microtitre plates for AST and MIC analysis. Plates are supplied either in pre-determined combinations and concentrations of antibiotics, or in customised combinations and concentrations. The Etest™ system from AB Biodisk uses a predefined gradient of antibiotic concentrations on a plastic strip. To determine MIC values, strips

are placed them on the surface of an inoculated agar plate and the inhibition ellipse formed after incubation intersects the graduated strip at the MIC value.

Although these systems may be effective and reliable for AST, they are prescriptive, static and inflexible, even when customisation is offered. A user who wishes to test a particular panel of antibiotics and/or concentrations which are not available in standard form has two options. First, they can order a customised product, which is cumbersome and wastes time. Second, they can partially use several available products, with the parts combining to give the desired panel, but this wastes the antibiotics and/or concentrations which are not used. Furthermore, users who need to run a variety of different tests on different samples have to maintain a large stock of standard products and reagents.

The prior art systems are therefore not well suited for AST work where a user might wish to depart from the standards supplied by manufacturers. There is thus a need for improvements to allow dynamic and customisable AST work to be performed. Thus the invention provides "random access antimicrobials" (RAA), which is the opposite of the pre-defined products of the prior art. Rather than prescribe to an end user the antimicrobials and concentrations which are available, "random access antimicrobials" dynamically allows them to prepare customised panels of chosen antimicrobials at chosen concentrations. The individual needs of individual workers are therefore satisfied.

The invention provides a RAA apparatus for preparing a customised panel of antimicrobials comprising a plurality of antimicrobials, each at a plurality of concentrations, wherein the apparatus comprises:

(a) a plurality of antimicrobial stock solutions;

(b) a dilution solution;

(c) a volumetric dispenser;

(d) an input device where a user can choose (i) which of the plurality of antimicrobials are to be included in the panel and (ii) the concentrations of the antimicrobials to be included in the panel; and

(e) a central processing unit (CPU) which, based on data from (d), controls (a), (b) and (c) to prepare the panel.

The invention also provides a RAA process for preparing a customised panel of antimicrobials comprising a plurality of antimicrobials, each at a plurality of concentrations, from a supply of a plurality of antimicrobial stock solutions, wherein the process comprises the steps of:

(a) extracting a predetermined volume of one of the antimicrobial stock solutions;

(b) diluting the extracted solution with a predetermined volume of a dilution solution, to give a diluted antimicrobial solution of a predetermined concentration; and

(c) repeating steps (a) and (b) until a plurality of diluted solutions of a plurality of antimicrobial stock solutions have been prepared.

The RAA apparatus and process require a supply of antimicrobial stock solutions of known concentrations. These solutions are diluted to give antimicrobial solutions of concentrations chosen by a user. Typical antimicrobials for use with the RAA invention are the same as those described above. The plurality is preferably at least 4 (*e.g.* 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50 or more). As an alternative to including solutions of antimicrobials, or as an upstream part of the preparation of solutions, the RAA apparatus and process may use dehydrated antimicrobials that are reconstituted to give solutions (either to give stock solutions for dilution to a concentration of interest, or reconstitution from dehydrated material direct to the concentration of interest without an intermediate stock solution phase). It is also possible to dilute dehydrated antimicrobials with a dehydrated carrier to give a random access panel of dehydrated antimicrobials which, when reconstituted, will give solutions of the desired concentrations.

It is possible to use more than one stock solution of a single antimicrobial, each having different concentrations, and this is particularly useful where a wide range of concentrations is required. For example, the apparatus might include a 100mg/ml and a 1mg/ml stock solution of the same antimicrobial. For preparing solutions with a concentration between 1-100mg/ml, the more concentrated stock solution will be diluted, and for preparing solutions of <1mg/ml, the less concentrated stock solution will be diluted.

The stock solutions may include a single antimicrobial each or they may include mixtures of antimicrobials. Furthermore, where stock solutions include a single antimicrobial, antimicrobials may be combined after being taken from the stock solutions in order to prepare dilutions of mixed antimicrobials.

The antimicrobial solutions comprise antimicrobials in aqueous media. The dilution solution will generally be the same medium as used in the stock solution which is being diluted. If an antimicrobial stock solution is dissolved in a phosphate buffer, for instance, then the dilution solution will also be a phosphate buffer. Furthermore, it will generally be the same phosphate buffer (*i.e.* same pH *etc.*). Dilutions may be made using the growth broth or medium to which the antimicrobial is going to be used. If the various antimicrobial stock solutions are based around different media, it is preferred that there are corresponding dilution solutions. Distilled water may be used as the diluent.

The dilution solution is used to dilute an antimicrobial stock solution to a predetermined concentration, with dilution involving mixing a known volume of stock solution with a known volume of dilution solution. To deliver these known volumes for mixing, the RAA invention uses a volumetric dispenser *i.e.* a device which can dispense a specific volume of liquid.

In one embodiment, the RAA invention uses a single volumetric dispenser, which is used to extract and dispense material from both the antimicrobial stock solutions and the dilution solutions, with mixing taking place either within the dispenser (*i.e.* the dispenser extracts both antimicrobial and dilution solutions without dispensing in between) or in a mixing vessel (*i.e.* the dispenser extracts

antimicrobial and dilution solutions separately and deposits them into the same vessel, where they are mixed). In the latter case, the same dispenser may also be used to transfer the solutions after mixing. In another embodiment, which will typically require fewer moving parts, the RAA invention uses a plurality of volumetric dispensers, each being given its own task. For example, each stock solution and dilution solution could have its own volumetric dispenser. Intermediate embodiments are also possible *e.g.* where a first volumetric dispenser is shared by the antimicrobial stock solutions and second is used for dilution solution(s), *etc.*

Because the RAA invention allows user-defined concentrations of antimicrobials to be selected, a variety of dilution factors may be required. It is thus preferred to use dispensers which can deliver more than one different volume of solution. However, some embodiments of the RAA invention may be able to use fixed volume dispensers *e.g.* if serial dilutions are required.

For any single antimicrobial, the combination of stock solution(s), dilution solution(s) and volumetric dispenser(s) can preferably create a range of dilutions of from 1.1x to 1000x (relative to the most concentrated stock solution), and can more preferably create a range of dilutions of from 2x to 128x. Within the range, it is preferred that at least 4 different final diluted concentrations can be prepared (*e.g.* 5, 6, 7, 8, 9, 10, 15, 20, 25, 30 or more). A power-2 series of dilutions is convenient *e.g.* 2, 4, 8, 16, 32, 64 fold dilutions.

A key aspect of RAA is the ability of a user to choose (i) the antimicrobials which are to be included in a panel and (ii) the concentrations of the antimicrobials to be included. Thus the apparatus of the RAA invention includes an input device which allows the user's choice to be entered. The input device will typically be based around buttons, switches, a keyboard or a pointer. The user's choices are relayed from the input device to the CPU which, based on the user's choices, controls the dilution of antimicrobial stock solutions, using the dilution solution(s) and volumetric dispenser(s), to create the desired panel of antimicrobials.

The panel of antimicrobials can take various forms. In one embodiment the panel might comprise a single solid support comprising (*e.g.* by impregnation, or by application to its surface) a plurality of "spots", with each "spot" including a specific antimicrobial at a specific concentration. Ten antimicrobials at ten concentrations each would therefore give a support comprising 100 spots. In a similar embodiment, the panel might comprise a plurality of supports, with each support containing a single antimicrobial but at different concentrations. Ten antimicrobials at ten concentrations might therefore be embodied as ten supports, each comprising ten spots. Of course, it could also be embodied as twenty supports, each comprising five spots, *etc.* Furthermore, the panel might comprise a plurality of supports, with each support containing a single antimicrobial at a single concentration (*e.g.* an antimicrobial disc). Ten antimicrobials at ten concentrations might therefore be embodied as 100 separate supports. Thus the RAA invention allows a customised panel of Kirby-Bauer-type discs to be prepared.

In another embodiment, the panel might comprise a collection of antimicrobial solutions, each with a different antimicrobial at a different concentration. The panel might be presented as individual solutions in individual containers, or as individual solutions in a single container (*e.g.* in individual wells of a microtitre plate). Intermediate positions are also possible *e.g.* a number of microtitre plates, each containing one antimicrobial at its various concentrations.

The panel need not exist simultaneously. For example, a member of the panel might be used soon after its preparation, before the remaining members of the panel have been prepared. Similarly, a single antimicrobial might be prepared in various concentrations and then used, before the next antimicrobial has been processed. Whilst the panel might never exist in a simultaneous tangible form under such circumstances, it does exist in the context of the overall procedure.

The apparatus of the RAA invention may comprise a multi-purpose display. This can be used to display a list of antimicrobials available within the apparatus. It can also be used to display available final concentrations. The display and the input device may be part of the same computer.

Central to RAA is that a predetermined set of concentrations of any particular antimicrobial is not imposed on a user. However, this does not mean that the user must have a limitless choice of the concentrations which may be prepared. For example, the apparatus may impose minimum and maximum dilutions (*e.g.* a dilution of 1.0001-fold or of $10^{10}$-fold may not be helpful), or it may restrict the user to choosing from a list of specific dilution factors based on the properties of the volumetric dispensers being used (*e.g.* 2x, 3x, 4x, 5x *etc.*). The apparatus may impose a maximum number of dilutions for any given antimicrobial (*e.g.* 1000 different dilutions of the same antimicrobial may not be helpful).

Although the apparatus is not prescriptive, it may offer suggestions. For example, it may suggest a predetermined range of concentrations for any specific antimicrobial. It may also suggest pre-determined individual values within the range. It may allow the user to select a range and then suggest individual values within the range.

The apparatus may monitor the available quantities of stock and dilution solutions. Based on such information, it may also be able to prompt a user that reagents are nearly exhausted.

The RAA apparatus may, in addition to antimicrobial dilution capability, comprise AST and MIC testing capability. Thus the antimicrobial dilution aspect of the invention can advantageously be incorporated into existing AST and MIC systems.

***Definitions***

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The term "about" in relation to a numerical value *x* means, for example, $x \pm 10\%$.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "antibody" includes any suitable natural or artificial immunoglobulin or derivative thereof. In general, the antibody will comprise a Fv region which possesses specific antigen-binding activity. This includes, but is not limited to: whole immunoglobulins, antigen-binding immunoglobulin fragments (*e.g.* Fv, Fab, $F(ab')_2$ *etc.*), single-chain antibodies (*e.g.* scFv), oligobodies, chimeric antibodies, humanized antibodies, veneered antibodies, phage-displayed antibodies, *etc.*

## BRIEF DESCRIPTION OF DRAWINGS

Figures 1 and 2 are a schematic plan view and perspective view, respectively, of the working parts of an apparatus of the invention.

Figure 3 shows a stack pack of the antimicrobial strips of Figure 4.

Figure 5 shows reaction substrates used in the apparatus.

Figure 6 shows the overall scheme of the invention.

Figure 7 shows the operation of rehydration carousel (55).

Figure 8 shows the benefits of parallelism using the apparatus of the invention, and Figure 9 shows the maximum throughput per day (samples per day) depending on the number of antimicrobials which are tested by AST.

## MODES FOR CARRYING OUT THE INVENTION

Figures 1 and 2 show the operational components of an apparatus (10), which is based around large main carousel (15). The apparatus (10) has a sample carousel (20) which can hold up to twenty patient samples (21) in 2-50ml bottles (22). A sample extraction mechanism (25) contains a pipetting system which can extract material from a bottle (22) and transfer it to reaction substrates (40) on carousel (15). The apparatus (10) also has an antibody carousel (30) which can hold up to sixteen 50ml bottles (32) of different IMS particles (31), suspended in a bacteriostatic preservative. An antibody transfer mechanism (35) can transfer IMS particles (31) from bottles (32) to wells (42) in reaction substrates (40).

Microorganism identification and testing take place in the wells (42) of reaction substrates (40) (Figure 5) while they are arranged around main carousel (15). The relative dimensions of substrates (40) and carousel (15) mean that substrates (40) can be radially arranged in two concentric rings. For convenience, one ring (*e.g.* the outer row) can be used for identification reactions while the other can be used for AST reactions. Sterile substrates (40) enter the carousel from substrate storage (48), where they are stacked; they exit the carousel (15) at waste removal station (90) where they are be disposed of into a waste storage facility (92) which includes strong bleach. The substrates are transparent such that luminescent reactions can be detected within wells (42).

An antimicrobial storage carousel (50) is located inside the main carousel (15) which can hold up to twelve sources (52) of different antimicrobials. Each source (52) contains a stack of dehydrated

antimicrobial strips (53) (Figure 3). Each strip (53) contains eight wells (54), and in a single strip (53) each well (54) contains the same antimicrobial (Figure 4) in dehydrated form such that, when rehydrated to the same volume, each well (54) contains a different concentration of the antimicrobial. The strips (53) are sealed for storage, and when a particular antimicrobial is required the top strip (53) of a stack is removed and the seal is peeled off (see arrows in Figure 3).

Around the antimicrobial storage carousel (50) is an antimicrobial rehydration carousel (55), a transfer mechanism (56) and an antimicrobial rehydrator (57) which are used to rehydrate the dehydrated antimicrobials within the wells (54) of strips (53). An antimicrobial transfer mechanism (58) transfers rehydrated antimicrobials to reaction wells (40) on the main carousel (15).

The apparatus includes two washing stations (61, 62) where reaction substrates can be washed.

The apparatus includes two luminometers (70, 80) which are used for detecting the results of microorganism identification assays (70) and microbial testing assays (80).

The apparatus has a magnetic extractor (72) containing high-strength permanent magnets. It also has an extracted sample rehydration mechanism (74).

The apparatus includes a supply of ≥50ml sterile BHI broth for microorganism culture, ≥700ml PBS for washing, ≥25ml luminescence AK assay reagents, disinfection solution (*e.g.* ethanol) for cleaning the apparatus, a waste bottle, and a luminometer calibration reagent. Appropriate dispensing systems are also included. The broth and luminescence reagents are stored in a refrigerator within the apparatus. The apparatus also includes an electric heating element and an air blower for maintaining microbial analyses at 37±2°C and for pre-heating reagents prior to their use. Excess air within the apparatus is vented via a HEPA filter.

The apparatus (10) is covered in a suitable case measuring about 70x55x100cm. Removable parts of the case give access for antimicrobial loading, addition of further reaction substrates (40), addition of extra IMS reagents, introduction of samples (21), access to the waste storage (92), *etc.* The use of proper covers also helps to control evaporation, which is important because of the small volumes involved in the analyses. A keyboard and display (and optionally a mouse) are also available externally.

During use, biological samples (21) are loaded into bottles (22) and placed in sample carousel (20). Additional bottles can be loaded to empty spaces as the procedure progresses, and this is particularly important when an sample must be analysed on an urgent basis.

Main carousel (15) indexes between fixed positions once every 60 seconds in order to move reaction substrates (40) into position for the next operation in the analysis procedure. At the start of the procedure, when carousel (15) is at angle zero, an 8-well reaction substrate (40) is loaded from storage (48) onto the outer row of carousel (15). The carousel (15) rotates until reaction substrates (40) are beneath antibody transfer mechanism (35). Immunomagnetic beads (31) specific for a microorganism of interest (*e.g.* specific for *N.gonorrhoeae*) are dispensed by the mechanism (35)

into reaction wells (42). At wash station (62), magnets (72) are inserted next to substrate (40), and the liquid contents of wells (42) are aspirated. A wash solution is added and the magnet (72) is removed, thus re-suspending beads (31). This washing procedure is repeated three times, with the final re-suspension using growth broth rather than wash solution.

The wells (42) are washed at wash station (62) to remove preservative from the beads (31).

Carousel (15) continues to rotate until the substrate (40) is beneath sample extraction mechanism (25). A pipette in the mechanism (25) extracts 8 unit volumes of sample (21) from bottle (22) and transfers one unit (100µl each) into each well in substrate (40).

Carousel (15) continues to rotate for 10 minutes, allowing the IMS beads (31) and sample (21) to interact in wells (42). The wells (42) are then washed at wash station (61) to leave bead/microorganism complexes. BHI broth is dispensed into the wells at dispenser (74) and the complexes are temporarily stored in wells (42) prior to microorganism identification.

Carousel (15) continues to rotate to luminometer (70). ADP and lysis buffer are added to the eight wells (42) in series, from the inside of carousel (15) to the outside. After 5 minutes of incubation at a fixed temperature, luciferase is added and, after a further 2 minutes, luminescence within each well (42) is assessed. To minimise stray signal, the luminometer (70) receives light from a single well. As each well is analysed separately, and as the luminescence reaction is time-dependent, the individual wells are dealt with in the same order as when lysis reagent was added (*i.e.* from inside to outside). Furthermore, the time between adding the lysis reagent to each well (42) is the same as the time required for reading the result from each well (42) within luminometer (70).

For a typical identification assay, carousel (15) rotates in a single direction in 43 steps, with each step taking 60 seconds, and with results of the AK assay being read after step 43:

| Time (s) | Duration (s) | Step | Action | Carousel angle (°) |
|---|---|---|---|---|
| 0 | 60 | 1 | Place substrate (40) onto carousel (15) | 0.00 |
| 60 | 240 | 2-5 | — | 2.16 |
| 300 | 60 | 6 | Transfer IMS beads (31) | 10.78 |
| 360 | 240 | 7-10 | — | 12.93 |
| 600 | 60 | 11 | Wash preservative off IMS beads (31) | 21.56 |
| 660 | 240 | 12-15 | — | 23.71 |
| 900 | 60 | 16 | Transfer sample (21) to substrate (40) | 32.34 |
| 960 | 600 | 17-27 | Incubate sample (21) and IMS beads (31) | 34.49 |
| 1560 | 60 | 28 | Wash IMS beads (31) | 56.05 |
| 1620 | 240 | 29-32 | — | 58.20 |
| 1860 | 60 | 33 | Add BHI broth to substrate (40) | 66.83 |
| 1920 | 240 | 34-37 | — | 68.98 |
| 2160 | 60 | 38 | Add ADP and EDTA reagents | 77.60 |
| 2220 | 300 | 39-42 | Incubate for lysis and AK release | 79.76 |
| 2520 | 60 | 43 | Dispense bioluminescence reagents & read | 90.54 |

A positive signal in the bioluminescence assay indicates that the original sample (21) contained the microorganism for which IMS beads (31) are specific *e.g.* that the sample contains *N.gonorrheae*. If a positive result is achieved, an AST assay is performed on the extracted microorganisms.

A further reaction substrate (40) is added to carousel (15), but this time on the inner row. The same process of IMS extraction is followed as described above, and the first 32 steps of the AST procedure are identical to those of the identification procedure. This common approach reduces complexity and cost of the instrument, and relies on the relevant transfer mechanisms being able to service both concentric tracks of carousel (15). Rather than re-suspend bacteria with BHI broth at (74) after step 32, however, antimicrobials are added instead using mechanism (58).

While IMS extraction is proceeding, therefore, one or more antimicrobial strips (52) is/are transferred from storage carousel (50) to rehydration carousel (55) by mechanism (56). After rehydration with water by rehydrator (57), antimicrobials are allowed to rotate for 5 minutes in carousel (55) before being transferred by pipette mechanism (58) to reaction wells (40). Used antimicrobial strips (52) are then discarded. Each strip offers eight concentrations of antimicrobial (Figure 4). Depending on the assay being used, one or more of these concentrations will be used (*e.g.* for a three point MIC, only three wells per strip (52) will be rehydrated for use). The rehydration carousel (55) operates on a 30 second index period, and its operation is shown in Figure 7.

As described above, antimicrobials are transferred into reaction wells (42) for the AST assay. Carousel (15) continues to rotate for 2 hours until the substrate (40) enters AST luminometer (80), where the effect of the antimicrobials on microorganisms within reaction wells (42) is determined by the same adenylate kinase/luciferase reaction as used for microorganism identification.

For a typical AST assay, carousel (15) rotates in a single direction in 167 steps, with each step taking 60 seconds, and with results of the AK assay being read after step 159:

| Time (s) | Duration (s) | Step | Action | Carousel angle (°) |
|---|---|---|---|---|
| 0 | 60 | 1 | Place substrate (40) onto carousel (15) | 0.00 |
| 60 | 240 | 2-5 | — | 2.16 |
| 300 | 60 | 6 | Transfer IMS beads (31) | 10.78 |
| 360 | 240 | 7-10 | — | 12.93 |
| 600 | 60 | 11 | Wash preservative off IMS beads (31) | 21.56 |
| 660 | 240 | 12-15 | — | 23.71 |
| 900 | 60 | 16 | Transfer sample (21) to substrate (40) | 32.34 |
| 960 | 600 | 17-26 | Incubate sample (21) and IMS beads (31) | 34.49 |
| 1560 | 60 | 27 | Wash IMS beads (31) | 56.05 |
| 1620 | 240 | 28-31 | — | 58.20 |
| 1860 | 60 | 32 | Add antimicrobials | 66.83 |
| 1920 | 7200 | 33-152 | Incubate & agitate | 68.98 |
| 9120 | 60 | 153 | Add ADP and EDTA reagents | 327.66 |
| 9180 | 300 | 154-158 | Incubate for lysis and AK release | 329.82 |

| | | | | |
|---|---|---|---|---|
| 9480 | 60 | 159 | Dispense bioluminescence reagents & read | 340.60 |
| 9540 | 240 | 160-163 | — | 342.75 |
| 9780 | 240 | 164-167 | Flush to waste | 351.38 |

As shown, after the AST assay has been performed and carousel (15) continues to rotate, the contents of reaction substrates (40) reach waste removal station (90) where they are pushed off carousel (15) and they descend into waste container (92). An audible and/or visual signal is given when an analysis has been completed.

The whole procedure is controlled by Windows™ computer (95). The computer (95) controls the movement of the carousels, the transfer of reagents and the routing of substrates (40) *e.g.* such that antimicrobials are transferred to extracted microorganism samples for AST at the correct time. It also controls timing within the overall procedure *e.g.* so that an antimicrobial is incubated with a microorganism for the correct amount of time for an AST assay. It receives and transmits information to various controllers within the apparatus (10) by RS-232 serial links.

The computer (95) can also hold different protocols for different assays *e.g.* it can hold different routing and timing information for MIC assays and AST assays.

Furthermore, the computer (95) has the facility to make informed choices based on ongoing results. For example, if a sample tests negative for the presence of a particular microorganism (*e.g.* a luciferase assay is negative after extraction using *N.gonorrhoeae*-specific IMS beads) then the computer (95) will not perform an AST assay on the empty reaction substrates (40) from that extraction. Instead, it will transfer the substrates to waste removal station (90) and will continue with further assays. Furthermore, based on the results of the identification assay the computer (95) can control the choice and/or concentration(s) of antimicrobial(s) to be used in AST *e.g.* the computer (95) will not test penicillin against bacteria which are known to be penicillin-resistant, and will not test low concentrations of antimicrobials against microorganisms which are known to be resistant to such low concentrations. The computer (95) thus controls the overall procedure at a general level, but it can also control it at a specific level based on the results obtained with specific samples.

The computer (95) also allows parallelism to be achieved simply. In a basic procedure, a sample (21) will be subjected to a first microorganism extraction (*e.g.* for *N.gonorrhoeae*), followed by AST for that microorganism (if it is found to be present). A second extraction (*e.g.* for MRSA) will then be performed, followed by a second AST assay, *etc.*, and these steps will proceed in series. By this method, however, only one reaction will proceed on the carousel at any given time. The computer (95) allows parallel sample processing to be used. For example, while AST for *N.gonorrhoeae* is being performed the computer (95) can easily arrange for MRSA extraction of the same sample to take place. A whole series of identifications can therefore be run on the same sample at the same time. Moreover, identifications and AST analyses can be performed in parallel *e.g.* MRSA extraction could be performed while *N.gonorrhoeae* AST is taking place. Furthermore, while MRSA AST on a sample from one patient is taking place, the computer (95) can begin identification analysis of the

next sample (21) on carousel (20) from a different patient, *etc.* As shown in Figure 8, therefore, during an 8 hour operation period, the apparatus goes through three phases: (1) only identification analyses are occurring; (2) AST analyses begin and identification analyses continue; and (3) after the end of the final identification analysis while the final AST analysis is completed.

The degree of parallelism is evidently limited by the relative timings of steps within the procedure (a single AST assay takes significantly longer than a single identification), but the computer (95) can easily arrange the various reactions to make optimum use of available time. Even if full parallelism is not achieved, serial analysis can be vastly improved. For example, identification assays can be performed for ten samples (21) in series, but these analyses can overlap in time. The computer (95) can then initiate AST analysis for the same 10 samples, again in an overlapping serial manner.

In between the extremes of full parallelism and overlapping serial analysis, the computer (95) can arrange three or more serial identifications while a single AST is progress. If only one in three identification analyses are positive then, in fact, even this partial parallelism offers significant advantages in terms of saved time.

The ultimate throughput of the apparatus is defined by the number of AST analyses a user chooses per sample. Figure 9 is a graph of the throughput as a function of the number of antibiotics tested per sample during AST analysis. For each number, figures are shown for three operating modes: (1) the left column shows throughput where AST alone is performed (no identification required); (2) the middle column shows throughput where ID of a single specific organism is performed, followed by AST; (3) the right column shows throughput where multi-organism identification and AST are performed [NB: the left column is always slightly higher because there is no delay while the first identification assay is processed]. For six antimicrobials, therefore, the apparatus can process at least 100 samples per working day, whichever of the three operating modes is selected. As the number of antimicrobials per test is reduced, the throughput increases. For the first two operating modes, throughput is not at all affected by identification assays, and very high throughputs are possible; for the third mode identification becomes the limiting step, so increases are lower.

The use of a computer (95) for control also simplifies the use of a variety of extraction protocols. As described above, for example, the computer (95) can have mechanism (25) take enough sample (21) only for extraction and identification and, if a positive identification is achieved, can then have mechanism (25) take further sample for AST *i.e.* sample (21) is visited twice, and so two separate extractions are required. As an alternative, the computer (95) can arrange for different wells (42) in the same substrate (40) to be treated differently after extraction, using some wells (42) for identification purposes and others for AST *i.e.* sample (21) is visited only once, and only one extraction step is required. As a further alternative, the computer (95) can arrange to take one sample (21) from a bottle (22) and put it in two separate substrates (40), using one substrate (40) for identification and another for AST *i.e.* sample (21) is visited once, but two separate extractions are required. As the computer (95) tracks where individual samples and extracted samples are located

within the apparatus, and knows where and when further sample and/or extracted samples are required, any of these three protocols can be used without difficulty, depending on preference. In some situations, it is more efficient to perform two small IMS extractions rather than one "double" extraction, and using separate extractions also minimises the need for extended temporary storage of microorganisms.

To permit parallel and overlapping analysis, the computer (95) must evidently be able to track the location of any particular reagent, sample, antimicrobial, *etc.* within the apparatus. This can easily be achieved either by knowing the absolute positioning within the apparatus *e.g.* the computer (95) knows that the reaction substrate in the third position on carousel (15) is the temporarily-stored *N.gonorrhoeae*-specific extract of the sample (21) in the third bottle (22) in carousel (20), and it knows the positions on the carousel where substrates (40) have been removed for disposal and where new substrates (40) can thus be introduced from storage (48). The computer (95) must also be supplied with information about which reagents have been placed in which positions in the apparatus *e.g.* it must know which antimicrobials have been placed in which positions in carousel (50), and likewise for IMS bottles (32). These data can be input by hand, but can also be ascertained by the computer (95) automatically by means of labelling *e.g.* by barcode labelling of packs of antimicrobial strips (52). Labelling of individual reaction substrates (40), antimicrobial strips (52), sample bottles (22) and/or IMS bottles (32) also allow a further layer of tracking within the apparatus.

The computer (95) can also monitor remaining supplies of consumables (antimicrobial strips, solutions, media, IMS particles, AK assay reagents, *etc.*) and can alert a user when supplies are running low. In terms of replacing supplies, the computer may lock access to relevant reagents during critical stages in the procedure to prevent replenishment from interrupting analyses.

***Random access antibiotics (RAA)***

An apparatus is prepared, including stock supplies of four dehydrated antibiotics: amoxycillin, penicillin, ciprofloxacin and streptomycin. The apparatus also includes a supply of fresh BHI broth. The apparatus also has a mixing chamber. The apparatus also has an automated pipetting system which can move between the various stock supplies and the mixing chamber, attached to a computer. The apparatus also has a powder movement system for delivering fixed volumes of the dehydrated antibiotics into the mixing chamber. The apparatus also has four storage chambers. The apparatus also has a set of output vials.

The computer offers the user a choice of (a) on or more of the four antibiotics and (b) one or more of ten fixed antibiotic concentrations (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 mg/ml). The user chooses these according to their needs and then confirms their choice. The computer then controls the pipetting system and the powder movement system to prepare stock solutions of the chosen antibiotics in BHI broth, with each stock solution stored in a separate storage chamber. The computer then controls the pipetting system to dilute the stock solutions with BHI broth to the chosen dilutions, with each final dilution being presented in a separate output vial.

If a user chooses concentrations in the 1-32 mg/ml range (*e.g.* 1, 2, 4, 8, 16 and 32 mg/ml) then the computer performs two separate procedures. First, a 32mg/ml stock solution is prepared, and this is delivered into output vials at undiluted strength and then at two serial two-fold dilutions. Second, a 4mg/ml stock solution is prepared, and this is delivered into output vials also at undiluted strength and at two serial two-fold dilutions. Thus the user receives the six desired concentrations, but prepared from two different stock solutions.

The vials can be used to perform AST on bacteria growing in BHI broth.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

**REFERENCES** (the contents of which are hereby incorporated by reference)

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## CLAIMS

1. An integrated apparatus for microbiological analysis of a patient sample, comprising: (a) a microorganism extractor, for extracting one or more microorganism(s) from the patient sample to give one or more extracted microorganism sample(s); (b) a temporary storage facility, for storing extracted microorganism sample(s); (c) a microorganism identifier, for identifying one or more specific microorganism(s) within the patient sample and/or within the extracted microorganism sample(s); (d) an antimicrobial tester, for determining the effect of one or more antimicrobial(s) on micro-organism(s) within extracted microorganism sample(s) and/or within the patient sample; (e) a thermostatically-controlled incubator for incubating extracted microorganism sample(s); (f) a timer; and (g) one or more sample routers, for routing: patient sample to the microorganism extractor; extracted microorganisms to the temporary storage facility; microorganisms from the temporary storage facility, patient sample or extracted sample to the microorganism identifier; and microorganisms from the temporary storage facility to the antimicrobial tester.

2. A process for analysing a patient sample, comprising the steps of: (a) identifying microorganisms present within the patient sample; (b) extracting one or more microorganisms from the patient sample, to give extracted microorganism sample(s); and (c) determining the effect of one or more antimicrobial(s) on extracted microorganism sample(s) or on the patient sample, wherein steps (a) and (b) are performed prior to step (c) but in either order.

3. The process of claim 2, wherein step (b) is performed multiple times.

4. The apparatus or process of any preceding claim, wherein the results of antimicrobial testing are reported in the same way as the results of microorganism identification such that a single detection mechanism can be used to determine the outcome of both assays.

5. The apparatus or process of any preceding claim, wherein the patient sample is obtained by processing material taken directly from a patient.

6. The apparatus or process of claim 5, wherein the material taken from the patient is blood, stools, swabs, mucous, or tissue.

7. The apparatus or process of any preceding claim, wherein the patient sample is urine, cerebrospinal fluid or a blood culture.

8. The apparatus or process of any preceding claim, wherein microorganism extraction uses an immunochemical technique.

9. The apparatus or process of claim 8, wherein microorganism extraction uses immunomagnetic separation.

10. The apparatus or process of any preceding claim, wherein 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more microorganisms are extracted.

11. The apparatus or process of any preceding claim, wherein the microorganism is a bacterium, a fungus or a parasite.

12. The apparatus or process of claim 11, wherein the microorganism is selected from the group consisting of: Staphylococci; Enterococci; Streptococci; Coliforms; Enteric organisms; Neisseria; yeasts; spirochaetes; and schistosoma.

13. The apparatus or process of any preceding claim, wherein identification of microorganisms is performed on an extracted microorganism sample.

14. The apparatus or process of any preceding claim, wherein microorganism identification is achieved by detecting the release of adenylate kinase after microorganisms are lysed.

15. The apparatus or process of claim 14, wherein microorganism identification is achieved using luminescence.

16. The apparatus or process of claim 15, wherein microorganism identification utilises a method involving lysing microorganisms in the sample, adding highly-pure ADP and a source of $Mg^{2+}$ ions to a sample and determining the amount of ATP produced from the ADP by adding luciferin and luciferase to the sample.

17. The apparatus or process of any preceding claim, wherein determining the effect of an antimicrobial includes antimicrobial susceptibility testing.

18. The apparatus or process of any preceding claim, wherein the technique is used to identify a minimum inhibitory concentration (MIC) value.

19. The apparatus or process of any preceding claim, wherein 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more antimicrobials are tested.

20. The apparatus or process of any preceding claim, wherein antimicrobials are tested at concentrations in the range from 0.05 to 50 mg/ml.

21. The apparatus or process of any preceding claim, wherein the antimicrobial is selected from the group consisting of beta-lactams, aminoglycosides, fluoroquinolones, sulfonamides, glycopeptides, carbapenems, azoles, oxazolidinones, macrolides, quinolones, and tetracyclines.

22. The process of any preceding claim, wherein the microorganism extractions used in step (b) are chosen on the basis of the identification results from step (a).

23. The process of any preceding claim, wherein the antimicrobials used in step (c) are chosen on the basis of the identification results from step (a).

24. A method for selecting an antimicrobial treatment for a patient, comprising the steps of: (a) sending a sample that has previously been taken from the patient for analysis to determine the effect of one or more antimicrobial(s) on micro-organisms in the sample, with optional identification of micro-organisms present in the sample; and (b) receiving for the results of step

(a), which results indicate one or more antimicrobial(s) that is/are effective against a micro-organism in the sample.

25. The method of claim 24, wherein the time which elapses between sending the sample in step (a) and receiving the results in step (b) is less than 24 hours.

26. A method for treating a patient with one or more antimicrobial(s), comprising selecting an antimicrobial by the method of claim 24 or claim 25, and further comprising the step of (c) administering to the patient one or more of the antimicrobial(s) indicated in step (b).

27. The method of claim 26, wherein the time which elapses between sending the sample in step (a) and administering the antimicrobial(s) in step (c) is less than 24 hours.

28. A method for selecting an antimicrobial treatment for a patient, comprising the steps of: (a) taking a sample from the patient; (b) sending the sample for analysis to determine the effect of one or more antimicrobial(s) on micro-organisms the sample, with optional identification of micro-organisms present in the sample; and (c) receiving for the results of step (b), which results indicate one or more antimicrobial(s) that is/are effective against a micro-organism in the sample.

29. The method of claim 28, wherein the time which elapses between sending the sample in step (a) and receiving the results in step (b) is less than 24 hours.

30. A method for treating a patient with one or more antimicrobial(s), comprising selecting an antimicrobial by the method of claim 28 or claim 29, and further comprising the step of: (d) administering to the patient one or more of the antimicrobial(s) indicated in step (c).

31. The method of claim 30, wherein the time which elapses between sending the sample in step (a) and administering the antimicrobial(s) in step (d) is less than 24 hours.

32. A process for performing antimicrobial susceptibility testing on a sample taken from a patient, wherein the time that elapses between taking the sample from the patient and providing the results of antimicrobial susceptibility testing is less than 24 hours.

33. A process comprising the steps of (a) taking a sample from a patient, (b) performing antimicrobial susceptibility testing on the sample to identify one or more antimicrobial(s) that is/are effective against micro-organisms present in the sample, wherein the time that elapses between taking the sample in step (a) and identifying an antimicrobial in step (b) is less than 24 hours.

34. A process for performing microbial identification and antimicrobial susceptibility testing on a patient sample, wherein microbial identification is performed without culture of microorganisms.

35. An apparatus for performing microbial identification and antimicrobial susceptibility testing on a patient sample, wherein microbial identification is performed without culture of microorganisms within the apparatus.

FIGURE 1

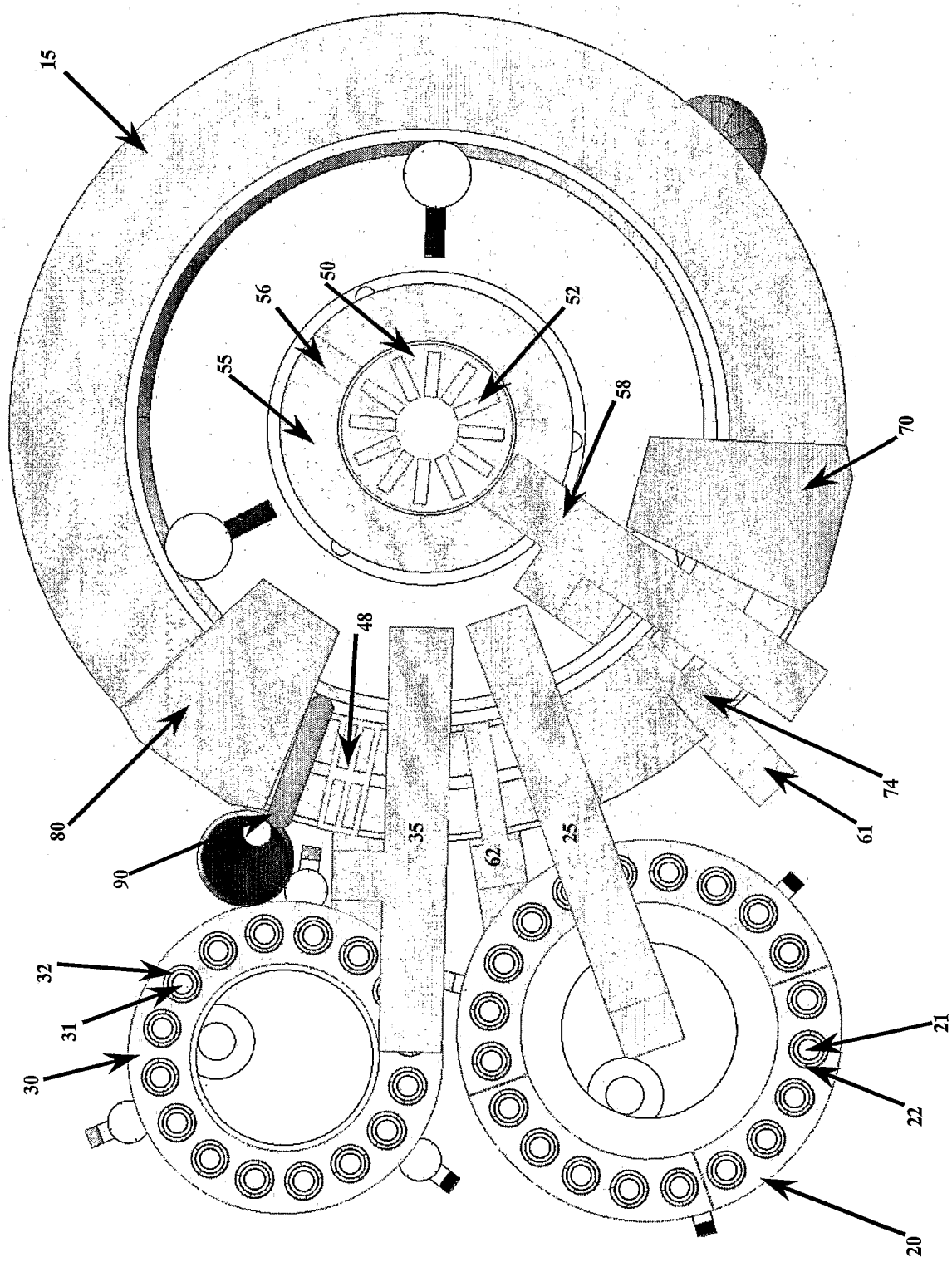


FIGURE 2

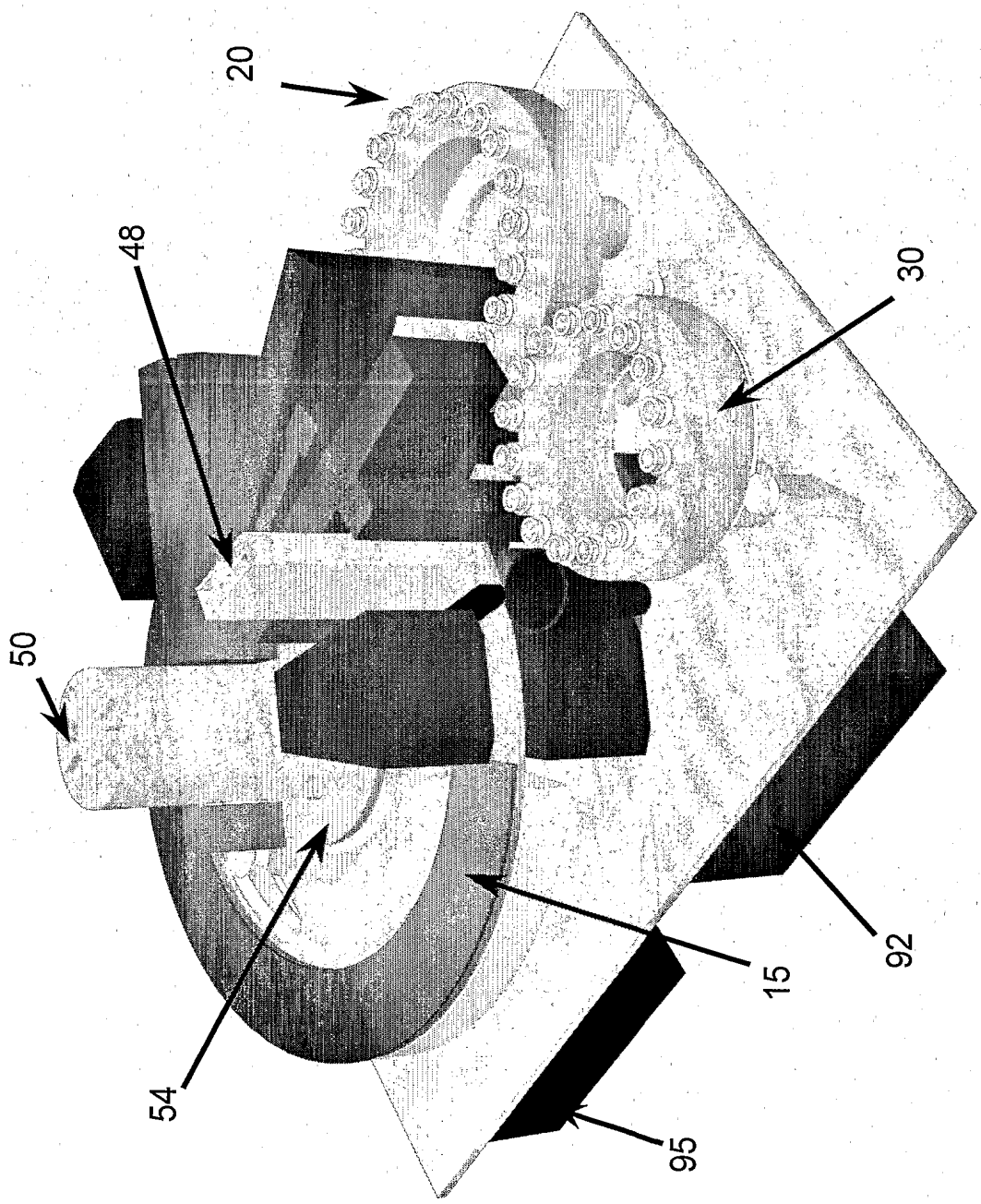


**FIGURE 3**

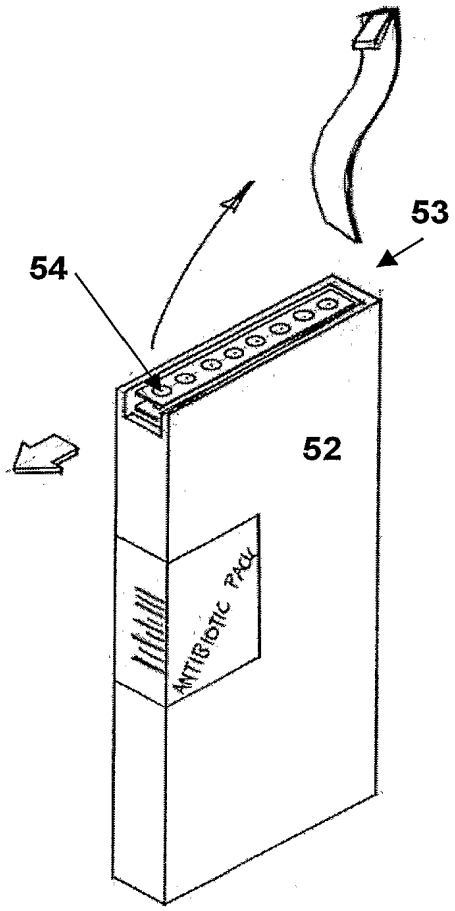



**FIGURE 4**

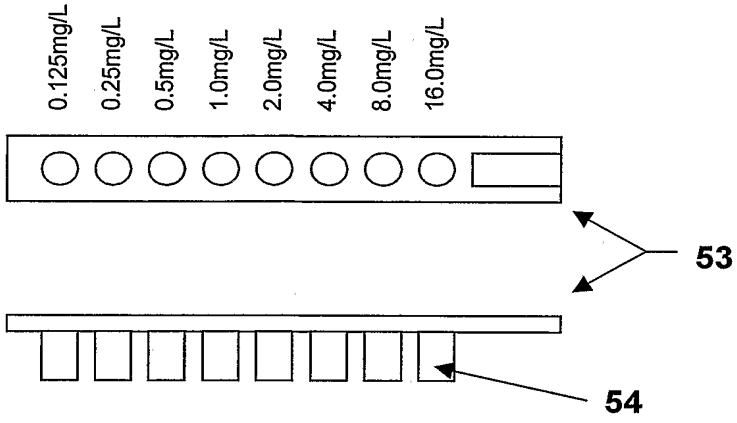



**FIGURE 5**

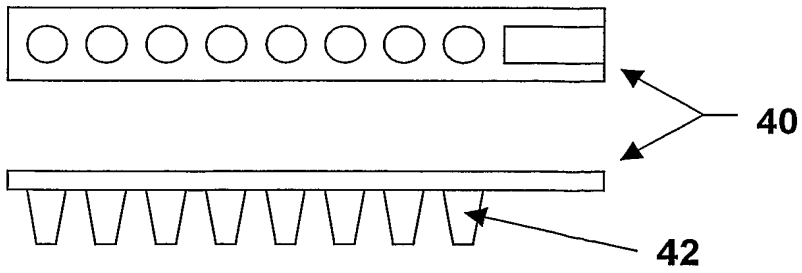


**FIGURE 6**

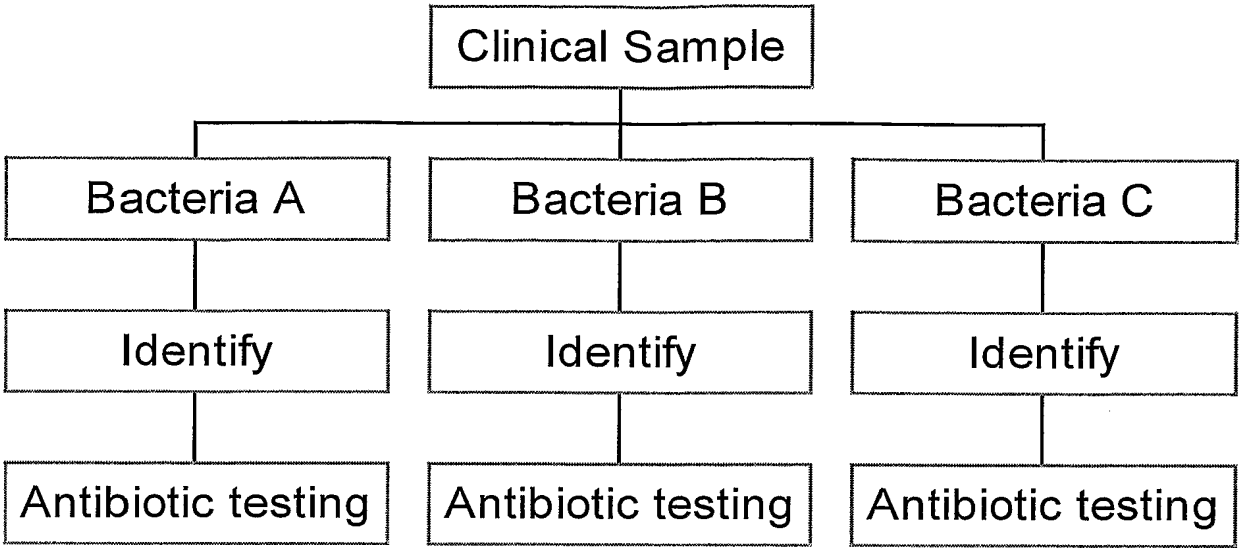



**FIGURE 7**

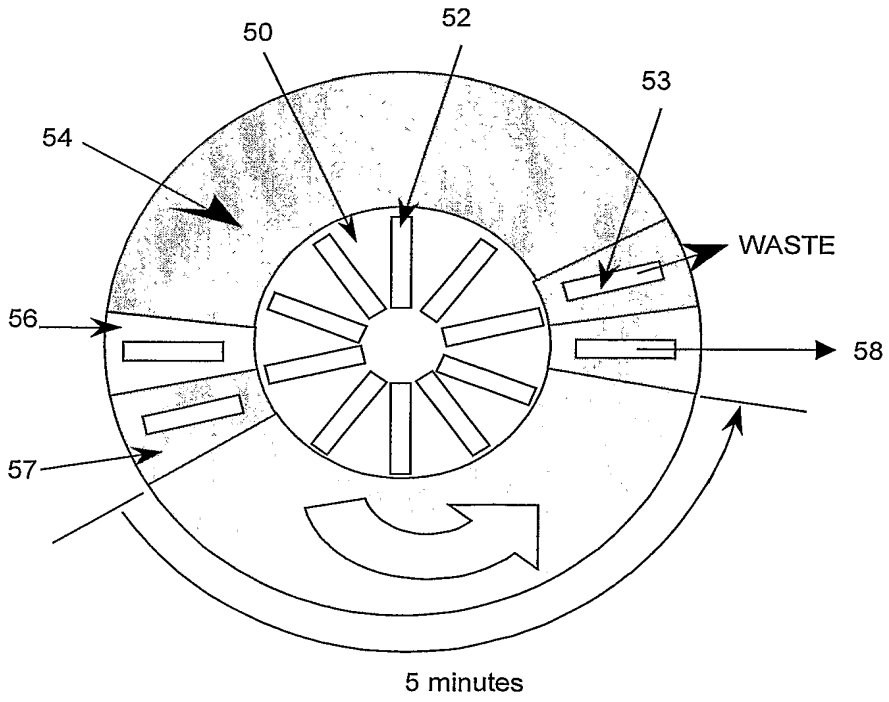


**FIGURE 8**

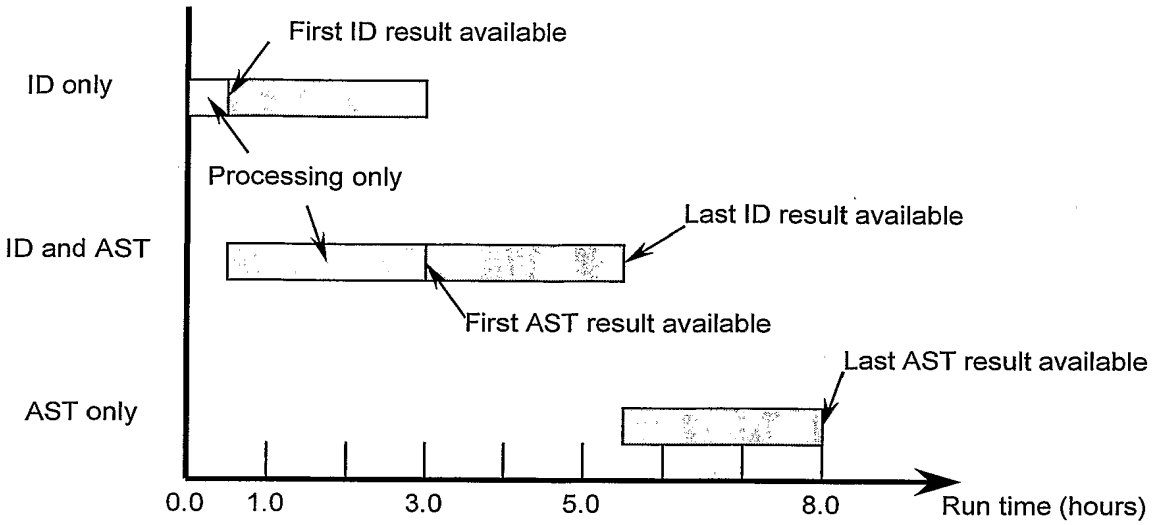
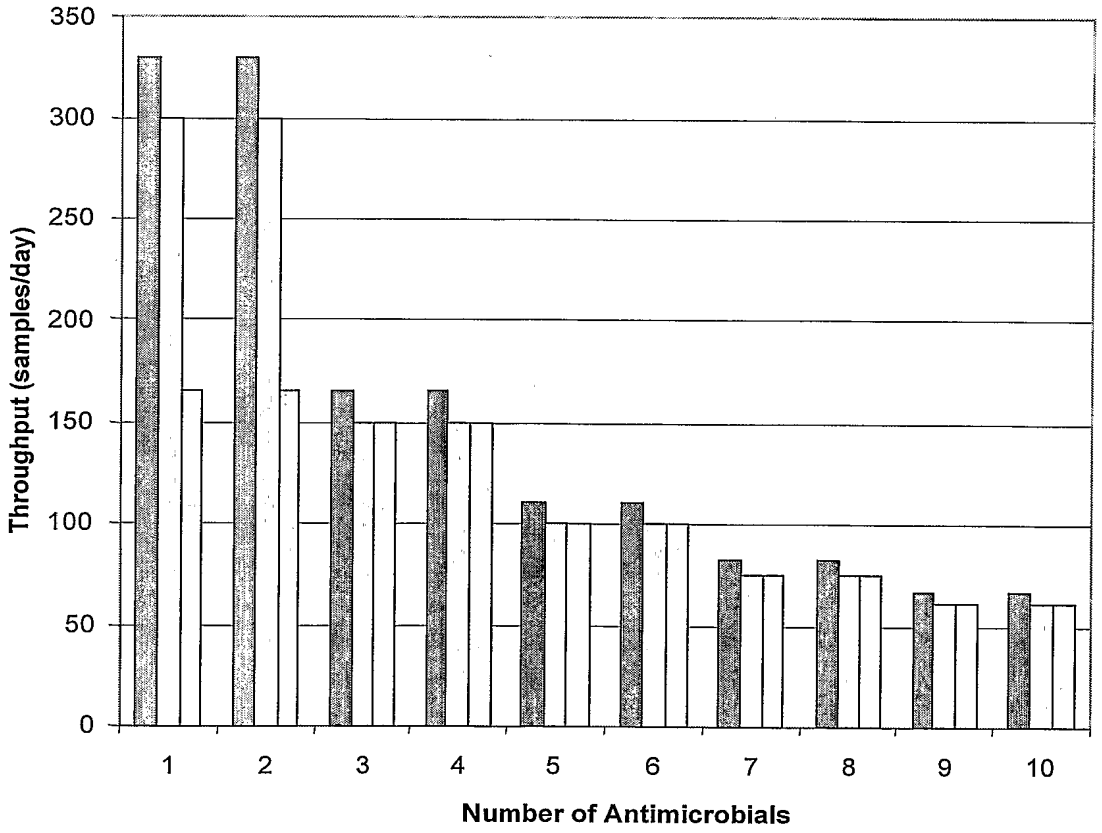



**FIGURE 9**



# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/001517

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12M1/24 C12M1/34 C12Q1/18 C12Q1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12M C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 95/14105 A (ROBERTS MICHAEL STEPHEN ; AQUACULTURE DIAGNOSTICS LIMITE (GB)) 26 May 1995 (1995-05-26) page 3, line 5 - page 4, line 35 | 1-35 |
| A | WO 98/53301 A (BECTON DICKINSON CO) 26 November 1998 (1998-11-26) the whole document | 1-35 |
| A | WO 00/67037 A (DADE MICROSCAN INC) 9 November 2000 (2000-11-09) the whole document | 1-35 |
| A | WO 99/37799 A (MURPHY MELANIE JANE ; PRICE RACHEL LOUISE (GB); SQUIRRELL DAVID JAMES) 29 July 1999 (1999-07-29) the whole document | 1-35 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 July 2004 | 12/08/2004 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Jacques, P |

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/001517

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | TU S -I ET AL: "Detection of immunomagnetically captured Escherichia coli O157:H7 by antibody-conjugated alkaline phosphatase" JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, vol. 26, no. 6, June 2001 (2001-06), pages 345-349, XP002290507 ISSN: 1367-5435 the whole document | 1-35 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2004/001517

**Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 26-31,33
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 26-31 and 33 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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Information on patent family members

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